# Phlebotominae spatial distribution associated with a focus of tegumentary leishmaniasis in Las Lomitas, Formosa, Argentina, 2002

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Las Lomitas, Formosa, Argentina, reported 96 cases of tegumentary leishmaniasis during 2002. The urban transmission was suggested although previous outbreaks were related with floods of the Bermejo river (BR) 50 km from the village. Phlebotomine collections were performed during March 2002 to define the spatial distribution of risk, together with satellite imaginery. The phlebotomine/trap obtained was 1679.5 in the southern BR shore, 1.1 in the periruban-rural environment and 2.3 in the northern Pilcomayo river marshes. Lutzomyia neivai was the prevalent species (91.1%) among the 2393 phlebotomine captured, and it was only found in the BR traps.

The other species were L. migonei (7.9%), L. cortelezzii (0.9%), and Brumptomyia guimaraesi (0.1%). The satellite images analysis indicates that the fishing spots at the BR were significantly overflowed during the transmission peak, consistent with fishermen recollections. This spatial restricted flood might concentrate vectors, reservoirs, and humans in high places. Therefore, both the spatial distribution of vectors and the sensor remoting data suggests that in Las Lomitas area the higher transmission risk it is still related with the gallery forest of the BR, despite of the urban residence of the cases. The surveillance and control implications of these results are discussed

Key words: leishmaniasis - Lutzomyia neivai - remote sensing - ecoepidemiology

Tegumentary leishmaniasis (TL) is endemic in Argentina above 28° latitude south. This area involves three different ecological regions: the transitional vegetation of the western (Yungas) and eastern (Paranaense) subtropical forests, and the savanna with xeric woods between them (Chaco) (Salomon 2004). Although the disease was known in the country for almost a century, the first recorded outbreak took place in 1984-1987 in the 'Yungas' region, due to *L. braziliensis* (Sosa Estani et al. 2000, Segura et al. 2000).

Formosa province, in the 'Chaco' region, reported to the National Surveillance System an average of 3.22 TL cases/year from 1955 to 1995, and 9.17 TL cases/year from 1996 to 2000, but the under-reporting was suggested for this period (Basulado et al. 2000). During the last decade most of the cases were diagnosed at the hospital of Las Lomitas, located in the central area of Formosa, with 85 TL cases between 1992 and 2001, 72.9% of them males, usually related with fishing activities during the periodic floods of the Bermejo river. Further, 99% of the phlebotomine sand flies captured in Las Lomitas surroundings in December 2001 were collected at the Bermejo river shore, and *Lutzomyia neivai* was the prevalent species (97.1%) (Salomon et al. 2002a). Thus, up to 2001 the epidemiological and entomological data indicated for Las Lomitas area the activities at the Bermejo river gallery forest as the main risk factor for TL transmission.

However, from January to July 2002 Las Lomitas Hospital reported 96 TL cases, with residence in Las Lomitas, and so the urban-periurban transmission was suggested, consistent with the urbanization trend of TL transmission in America (Campbell-Lendrum et al. 2001). Therefore, phlebotomine sand flies captures were performed during the peak of case reporting in order to determine the actual spatial distribution of risk, while environmental disturbances were looked for thorough satellite imaginery. The results are presented here and discussed in the frame of spatial transmission and outbreak origin hypotheses, remote sensing tools and appropriate control measures.

#### MATERIALS AND METHODS

*Studya area* - The study was conducted in Las Lomitas city (24°42'LS, 60°35'LW) and surroundings, department of Patiño, Formosa Province. The department (24,504 km<sup>2</sup>) is limited to the north by the Pilcomayo river marshes and to the south by the Bermejo-Teuco river. Patiño has 64,830 people, 10,354 of them in Las Lomitas city (Census 2001, National Institute of Statistics and Censuses). The climate is subtropical with a dry season, the landscape is typical of the Chaco ecological region: xeric thorn scrubs, palms, and overexploited woods of 'quebracho' *Schinopsis* and *Aspidosperma*.

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Sand fly collections - The capture sites were categorized according to the place and environment in 'Perirubanrural', 'Pilcomayo marshes', and 'Bermejo river' sites (Fig. 1). The periurban ones (n = 13) were selected taking into account vegetation, water streams, current cases, and supposed places of infection incriminated by the local population. The rural places (n = 5) were located up to 12 km from the city within wooded patches close to cases dwellings or in a deforestation front. The Pilcomayo marshes comprise capture stations from Las Lomitas to the northern border with the Republic of Paraguay (n = 7), in two directions: (a) provincial Road 32nd: 60 km from Las Lomitas, Fortín Soledad village in the peridomestic habitat of cases, and 48 km in a rural settlement (2 sites each); (b) provincial Road 28th: 37 km and 40 km in rural peridomestic environments, and km 45 km, 'La alcantarilla' in 'Bañado La Estrella' marshes, a fishing and recreational point with patches of palms and Aspidosperma trees. The Bermejo river sites (n = 2) were located 50 km from Las Lomitas to the southern border with Chaco province at two fishing spots, La Victoria and San Jorge, on the gallery forest of the river shore. Sand flies were captured from 17 to 8 with mini light traps CDC between March 10th and 16th 2002, each site two nights. The temperature range during the trapping nights was 24.3°C-28.7°C, the relative humidity 71-85%. Captures performed by the same method in two periurban, one rural and La Victoria Bermejo river sites in December 2001, already published (Salomon et al. 2002a), were included in the results for comparative purposes.

Sample processing and data analysis - All captured sand flies were stored dry until identification using the keys of Young and Duncan (1994) and Marcondes (1996). Fisher exact two-tailed test or  $\chi^2$  test were regarded as significant with associated probabilities  $\leq 0.02$  (CI 95%).

Satellite imaginery - Four satellite images of the study area (Path/Row 228/77) from two consecutive years were used. Two images were from dates with low Bermejo river flow (09/28/2000 and 15/09/2001), and the others from dates immediately after the annual flood (05/18/2001 and 05/21/2002). The former two were Landsat 5 TM and the

latter Landsat 7 ETM, all provided by Conae. Although the maximal and minimal flow of the Bermejo river recorded for both years were in March and October respectively, the images were the closest available to these dates. The images were georreferenced using satellite ephemeris and the nearest-neighbor method. The Band 5 (reflected midinfrared) was used in order to discriminate areas covered by the river (values of digital number-DN- between 0 and 22) and the surrounding land uncovered of vegetation (visual identification). The uncovered shores, data from May image minus that of September of the previous year, were regarded as a quantitative indicator of the flood area during March.

#### RESULTS

Phlebotomine captures in March 2002 produced 2393 sand flies in 10 out of the 27 sites sampled. *L. neivai* (*L. intermedia* complex) was the prevalent species (91.1%), *L. migonei* (7.9%), *L. cortelezzii* (0.9%), and *B. guimaraesi* (0.1%) were also found (Table). The abundance were significantly higher in the gallery forest of the Bermejo river than the Pilcomayo marshes basin or the periurban-rural sites: 1679.5, 2.3 and 1.1 phlebotomine/trap respectively. The species composition also differs among the three environments.

*L. neivai* was only collected in the Bermejo river associated vegetation, which it is also the unique environment with gravid females, *L. migonei* (15.7% of females), *L. neivai* (7.3%). Although no permanent human population is living there both sites on the river shore were usually visited for fishing, and the one with higher proportion of *L. neivai* was close to a water pumping station out of service.

Periurban-rural captures were consistently lower than Bermejo captures, sand flies were collected in 3 out of 13 periurban places and in 1 out of 5 rural sites. *L. migonei* and *L. cortelezzii* were represented by scarce individuals, but both species were present in almost all traps in the city vegetated surroundings that collected phlebotomine. The Pilcomayo marshes traps only produces *L.migonei*, the higher capture (11 individuals) in the peridomestic environment of a case located in Fortin Soledad village.

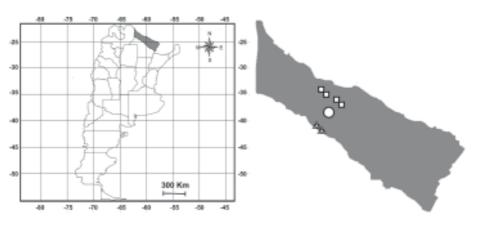


Fig. 1: phlebotomine capture stations, Formosa province, Argentina, March 2002. Las Lomitas periurban and rural sites (circle), Pilcomayo river marsh sites (squares), and Bermejo river sites (triangles).

Captures performed in December 2001 in the same area by mini light traps collected 966 individuals, *L. neivai* 98%, *L.migonei* 1.7%, and *L.cortelezzii* 0.3%; 99.7% of the overall capture was made in the Bermejo river shores, the remaining 4 *L. neivai* were found in peripheral sites of Las Lomitas city (Table). The captures at the Bermejo river site from December 2001 and March 2002 did not differ significantly either in abundance, species relative abundance or sex proportion of *L. neivai* and *L. migonei*. *B. guimaraesi* first report in Formosa province was only found in March 2002 Bermejo captures. On the other hand, periurban *L. neivai* was only collected in December 2001, while periurban *L. migonei* and *L. cortelezzii* were already reported in Las Lomitas previous captures with Shannon modified trap (Salomon et al. 2002a).

The satellite imaginery of the Bermejo river 50 km from Las Lomitas (Fig. 2) shows a larger area of uncovered land on the shore at the fishing spot in the May 2001-September 2002 images, than in May 2000-September 2001 ones. Therefore, these areas at this particular bend of the river were computed for each series (May minus September) and they were significantly different: 2000/2001-124.2 km<sup>2</sup>, and 2001/2002-710.1 km<sup>2</sup>.

#### DISCUSSION

Phlebotomine captures performed close to the Bermejo-Teuco river in Las Lomitas area during an 'urban' outbreak collected *L. neivai* as the prevalent species (>90%) with gravid females, followed by *L. migonei* and less *L. cortelezzii*. Thus, sand fly abundance and species composition within the gallery forest of the Bermejo river in the 'Chaco' region are the same as those found in the western hyperendemic area of TL transmission, in the foothills of the "Yungas" subtropical forest on the same Bermejo river basin (Salomon et al. 2004), and different from those found in the eastern subtropical forest (Salomon et al. 2002b). On the other hand, the perirubanrural and Pilcomayo marshes collections, with scarce in-

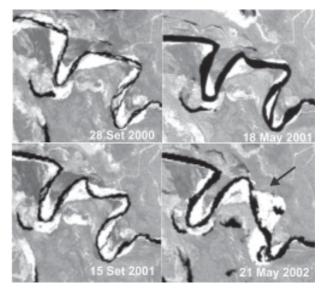


Fig. 2: Bermejo river shore, Las Lomitas area of influence (Provincial Road 28th south). Landsat 5 TM (09/28/2000, 15/09/2001) and Landsat 7 ETM (05/18/2001 and 05/21/2002). The arrow indicates the fishing site, in black the river course, in white the surrounding land uncovered of vegetation regarded as an indicator of the extent of the previous flood.

dividuals of *L. cortelezzii* and *L. migonei* resemble the captures reported up to 1950 in 'Chaco' landscapes of Formosa province where TL transmission was sporadic (Bejarano & Duret 1950). Historical collections also recorded for the province *L. shannoni* in San Hilario (Castro 1959), and *L. intermedia s.l.* in the Bermejo- Paraguay rivers confluence (Del Ponte 1960).

*L. intermedia* complex species (*L. intermedia* or *L. neivai*) and *L. migonei* have been incriminated as suspected vectors of *Leishmania braziliensis*, the former in domestic foci of Brazil, Paraguay, and Argentina (Rangel

TABLE

Phlebotomine captured with mini light trap (two nights) by species, sex (females/males), and site (only sites with sand flies) at different environments in Las Lomitas area, Formosa province, Argentina, March 2002. Captures performed during December 2001, already published (Salomón et al. 2002a) were also included

| Station          | Date  | Coordenates              | Ln      | Lm    | Lc  | Bg  | Total |
|------------------|-------|--------------------------|---------|-------|-----|-----|-------|
| Bermejo river    | 12/01 | 25°01'14"LS, 60°50'27"LW | 689/254 | 9/7   | 3/0 | 0   | 962   |
|                  | 03/02 | 25°01'14"LS, 60°50'27"LW | 717/268 | 11/8  | 3/0 | 0   | 1007  |
|                  | 03/02 | 25°01'12"LS, 60°52'62"LW | 581/615 | 83/50 | 9/6 | 1/0 | 1345  |
| Periurban- rural | 12/01 | 24°41'15"LS, 60°35'22"LW | 2/2     | 0     | 0   | 0   | 4     |
|                  | 03/02 | 24°41'15"LS, 60°35'22"LW | 0       | 3/2   | 0/3 | 0   | 3     |
|                  | 03/02 | 24°41'23"LS, 60°35' 9"LW | 0       | 6/2   | 1/0 | 0   | 9     |
|                  | 03/02 | 24°41'67"LS, 60°37'30"LW | 0       | 2/2   | 1/2 | 0   | 7     |
|                  | 03/02 | 24°43'14"LS, 60°35'07"LW | 0       | 1/0   | 0   | 0   | 1     |
| Pilcomayo basin  | 03/02 | 24°26'62"LS, 60°22'54"LW | 0       | 1/2   | 0   | 0   | 3     |
|                  | 03/02 | 24°24'55"LS, 60°20'05"LW | 0       | 1/0   | 0   | 0   | 1     |
|                  | 03/02 | 24°14'19"LS, 60°37'69"LW | 0       | 1/0   | 0   | 0   | 1     |
|                  | 03/02 | 24°08'97"LS, 60°41'40"LW | 0       | 9/2   | 0   | 0   | 11    |
| Total            |       |                          | 3128    | 202   | 28  | 1   | 3359  |

Ln: Lutzomyia neivai; Lm: L. migonei; Lc: L.cortelezzii; Bg: Brumptomyia guimaraesi.

et al. 1990, Salomon et al. 2004), the second one in peridomestic habitats that usually involves dogs and horses (Aguiar et al. 1987, Azevedo et al. 1990). Therefore, the spatial distribution of sand flies reported here suggest a relative risk much higher in the Bermejo river area than that in the urban or Pilcomayo area. Further, in a simultaneous epidemiological study (to be published elsewhere) the visit to the river risk sites differs significantly between cases and leishmanine negative controls (OR 0.59 < 2.29 < 8.99).

The TL cases recall the starting skin lesion between October 2001 and April 2002 with the mode and the median in March 2002. The seasonal distribution of the cases diagnosed in Las Lomitas hospital from 1992 to 2001, based on the probable date of infection was significantly associated with the average river flow (May-June) of the same year and the cumulative rainfall (January- May) of the previous year (Salomon et al. 2002a). However, neither the rainfall nor the Bermejo river flow showed any difference related to the 2002 outbreak. The 2001 January-May cumulative rainfall was 598.9 mm and it did not differ significantly from the 1990-2000 average 418.2 ( $\pm$  180.0) mm or the 2000 precipitation 510.1 mm (National Service of Meteorology, Argentine Air Force). Bermejo river, El Colorado hydrologic station on the other hand, the closest to the study area (26°20'00"LS, 59°21'49"LW, 78 masl), recorded flow over 1000 m<sup>3</sup>/s only from February to March, but these records did not differ significantly between the year 2000  $(1394 \pm 282,8 \text{ m}^3\text{/s})$ , 2001 $(1168 \pm 275,4 \text{ m}^3\text{/s})$  and  $2002 (1238 \pm 157, 2 \text{ m}^3/\text{s})$  (Sistema Nacional de Información Hídrica, National Hydrologic Network, EVARSA). Nevertheless, the satellite imaginery showed a focal overflow just at the fishing spots during the TL transmission period (Salomon et al. 2004). Unfortunately the images from March were unavailable due to cloudiness or technical problems, so the uncovered post-flood land in May next to the shores were used as an indicator of the extent of the previous flood, consistent with the fisherman recollections. This spatial restricted flood might concentrate vectors, potential reservoirs and humans in high places increasing both the parasite circulation, and the humanvector effective contact.

In conclusion, in Las Lomitas area the higher TL transmission risk it is still related with activities within the gallery forest of the Bermejo river, despite of the urban residence of the cases. However, the cases in Fortin Soledad (Pilcomayo river) and the presence of *L. neivai* in Las Lomitas city suggested a peridomestic cycle with sporadic cases or lower magnitude overlapped to the sylvatic one. In this scenario an spatial intervention against the vector in the urban area will have none or little impact on the TL incidence, even during the transmission peak, further when it was required during the peak of case diagnoses after the end of the vector season. Besides, from the sand fly ecology point of view the forest associated with the Bermejo river is a 'Yungas' subtropical forest corridor in the 'Chaco' xeric region, while the captures performed in Las Lomitas and Pilcomayo river better represents the 'Chaco' fauna.

Finally, the TL incidence in the area up to 2001 was fairly associated with climatic and hydrological regular

records, but the cases above average reported from Las Lomitas Hospital during the first half of 2002 are related with an overflow at a fishing point tracked only by satellite imaginery. Thus, remote sensing would be useful for microfocal surveillance of TL transmission, in space to conceive local adapted recommendations, and in time to active early warning systems. The Bermejo river basin could be surveilled at fishing points during the periodic overflows and each site categorized as sound, to be visited with personal protection, or to be avoided or banned. In the last ones areas the primary health care agents will be warned to perform active search of new TL cases. However, should be noted that the 2001 economic crisis turned recreational fishing and hunting to subsistence activities for an increasing number of people, and so the fulfillment of the recommendations will be dubious. Further, there are projects of deforestation and irrigation from the Bermejo river toward urban areas that will change the landscape and the sand fly abundance where L. neivai is already present. Thus, together with the surveillance and monitoring of the environment with remote sensing data, the impact on TL transmission should be included in the Environment Impact Assessment demanded to project developers in the endemic TL areas, in order to avoid an actual peridomestic cycle and urban-periurban outbreaks in the region.

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